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Metabolism of some Anionic Tallow-based Detergents by Sewage Microorganisms[1]

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A method in which the test detergent was the sole source of carbon was used to study the metabolism of several tallow-based detergents. These were tallow alcohol sulfates, long-chain ether alcohol sulfates, and esters of α-sulfo fatty acids. Sodium *p*-(1-methylundecyl)benzenesulfonate (LAS) was used as a reference material. The alcohol sulfates were the most rapidly and completely metabolized (96 to 99%), and one ether alcohol sulfate was 94% degraded. The other compounds were metabolized to the extent of 61 to 87%; LAS was 80% degraded. Except for the alcohol sulfates, loss of methylene blue activity (MBAS) occurred long before the chemical oxygen demand (COD) values had reached a minimum; with the alcohol sulfates, MBAS and COD decreased simultaneously.

The presence of anionic detergents has usually been determined by some modification of the methylene blue procedure. This method depends on the formation of a colored, chloroform-soluble complex by the detergent and methylene blue molecules. Loss of the complexing ability of detergents occurs when the carbon chains are shortened to about six to eight carbon atoms. This method can show greatly decreased amounts of detergent, or even none, when, in fact, a good part of the original material may be present as smaller molecules.

In 1963, M. S. Konecky, R. J. Kelly, J. M. Symons, and B. L. McCarty (Ann. Meeting Water Pollution Control Federation, 36th, Seattle, 1963) described a procedure (Esso Research Biodegradation Test) to determine the ability of microorganisms to utilize detergents as the sole source of carbon and energy. Detergent biodegradation under these conditions was measured by the decrease in chemical oxygen demand (COD) that results when the surfactant is metabolized. This procedure has now been used to test several tallow-based detergents.

Materials and Methods

Three types of detergents were tested: (i) esters of α-sulfo fatty acids (sodium methyl α-sulfostearate, sodium isopropyl α-sulfostearate, disodium 2-sulfoethyl α-sulfostearate, and sodium hexyl α-sulfopelargonate); (ii) tallow alcohol sulfates (sodium oleyl sulfate, sodium 9,10-dichlorooctadecyl sulfate, and hydrogenated tallow alcohol sulfates); and (iii) ether alcohol sulfates (sulfated polyoxypropylated hexadecanol, sodium octadecyloxyethyl sulfate, sodium hexadecyloxypropyl sulfate, and sodium 1-ethyl-2-hexadecyloxyethyl sulfate). These detergents have been studied by different methods under aerobic (2) and anaerobic (5) conditions. The individual isomer, sodium *p*-(1-methylundecyl)benzenesulfonate (LAS) was used as a reference material. We thank J. E. Shewmaker of the Esso Research and Engineering Co. for supplying this material.

The tests were carried out in 1-gal (3.8-liter) wide-mouth jars which contained 3 liters of deionized water, 10 mg of inoculum per liter, nutrient salts, and 40 mg of detergent per liter. Contents were stirred continuously by magnetic stirrers to insure adequate aeration. The jars, a food-industry type, had a curved bottom which kept the stirring bar centered over the magnetic drive.

The inoculum used was activated sludge from a sewage-treatment plant which treats mostly domestic sewage. "Disacclimation" to remove detergent already present was accomplished in a laboratory model of an activated sludge plant (4) which operated continuously for at least 1 week. Trout chow was used as the feed. The contents of each wide-mouth jar was inoculated with a slurry of bacterial sludge which resulted in a concentration of 10 mg per liter on a dry weight basis.

The stock solutions of nutrient salts (free of sulfates to permit possible use to follow sulfate ion formation from anionic detergents; J. E. Shewmaker, personal communication) had the following compositions.

Solution 1 contained, per liter: $Mg(NO_3)_2 \cdot 6H_2O$, 5.375 g; $Ca(NO_3)_2 \cdot 4H_2O$, 2.925 g; $Fe(NO_3)_3 \cdot 9H_2O$, 0.375 g; and $CoCl_2 \cdot 6H_2O$, 1.025 g. Solution 2 con-

[1] Presented in part at the Annual Meeting of the American Society for Microbiology, Los Angeles, Calif., 1–5 May 1966.

tained, per liter: $(NH_4)_2HPO_4$, 4.76 g; K_2HPO_4, 36.6 g; and KH_2PO_4, 31.1 g.

A 1-ml amount of solution 1, and a 5-ml portion of solution 2 were used per liter.

COD. COD was measured by a semimicro modification of the standard method of the American Public Health Association (1). Samples were centrifuged to remove particulate matter before analysis. Since the initial detergent concentration was 40 ppm, the COD values were multiplied by 40/theoretical COD, so that values are expressed as parts per million of detergent and can be compared with values for methylene blue active substance (MBAS).

MBAS. MBAS was determined on the settled samples by the method of Degens (3). The test period was 21 days. Samples were withdrawn from the bottles at intervals, usually daily, and analyzed by the procedures described above.

A blank and a control were used in each test series. The blank included reagents and organisms but omitted the detergents. The control was run with LAS as the reference compound.

Results and Discussion

Figure 1 shows a comparison of sodium isopropyl α-sulfostearate with LAS. The numbers are the dates the runs were started in 1965. For the isopropyl ester under disacclimation conditions, the rate of metabolism varied in the three runs for the first 7 days but thereafter decreased at a slow, steady, rate.

For LAS with fresh inoculum, the runs varied in rate and reached a COD of 10 ppm in 7 to 12 days. Disacclimation greatly decreased the rate of metabolism. Typical COD and MBAS curves with fresh inoculum are shown (bottom graph, Fig. 1). Values for MBAS dropped to zero in 6 days, but an unmetabolized residue (15 to 20% of the original detergent) persisted even after 2 weeks.

Figures 2–4 show the rate of metabolism of tallow-based detergents with disacclimated inocula. The low COD value for hydrogenated tallow alcohol sulfates (HTAS) at zero time (Fig. 2) was due to low solubility. Small increases in COD after an initial drop are attributed to the lysis of cells which releases soluble carbon into solution. Sodium oleyl sulfate and HTAS were very quickly and easily degraded, but the presence of the 2 chlorine atoms made sodium 9,10-

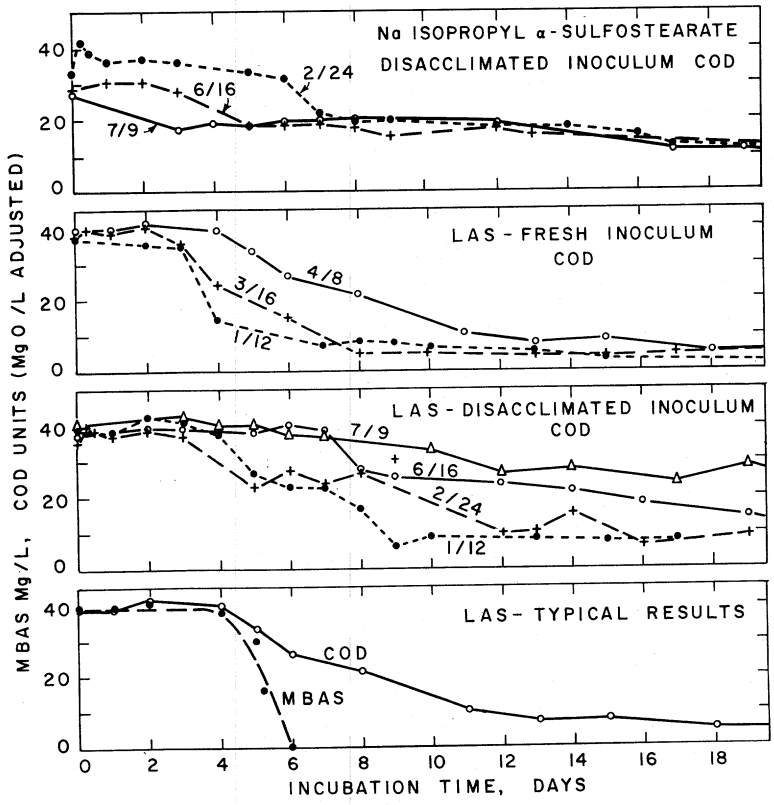

FIG. 1. *Effect of inoculum preparation on the metabolism of LAS and sodium isopropyl α-sulfostearate. COD units are adjusted to ppm of detergent. Numbers on the curves are the date the run was started, 1965.*

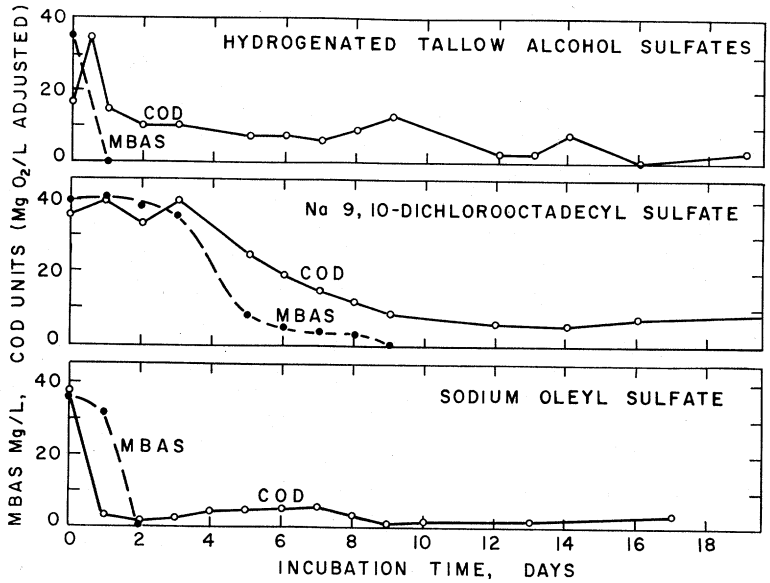

FIG. 2. *Metabolism of some tallow alcohol sulfates as shown by decrease in COD and MBAS. COD units are adjusted to ppm of detergent.*

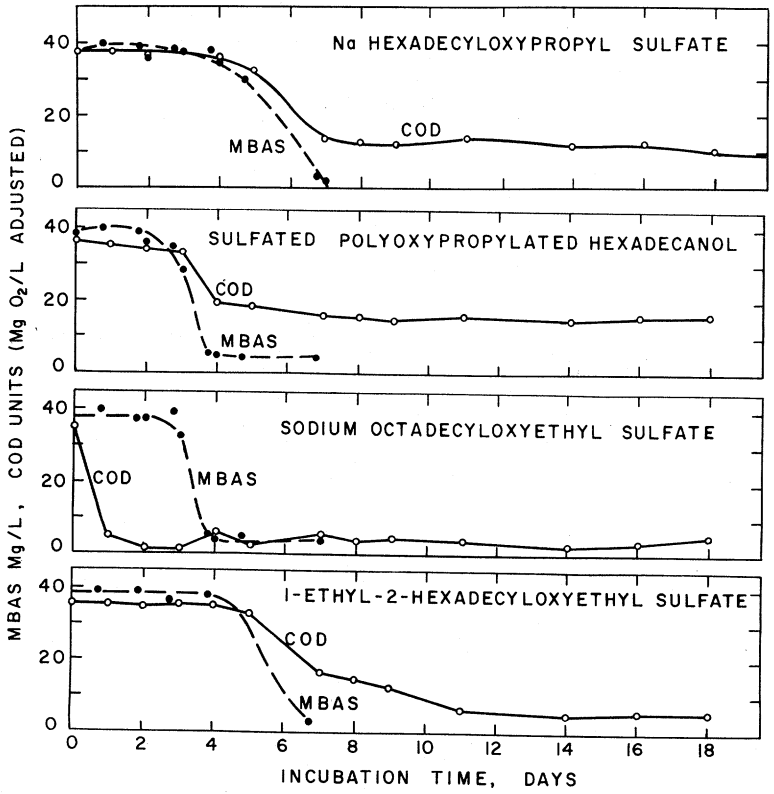

FIG. 3. *Metabolism of some ether alcohol sulfates as shown by decrease in COD and MBAS. COD units are adjusted to ppm of detergent.*

dichlorooctadecyl sulfate somewhat more resistant to attack and less completely metabolized.

Figure 3 shows the results with four ether alcohol sulfates: sodium hexadecyloxypropyl sulfate, $C_{16}H_{33}OCH_2CH(CH_3)OSO_3Na$; sulfated polyoxypropylated hexadecanol, $C_{16}H_{33}[OCH_2CH(CH_3)]_{2-3}OSO_3Na$; sodium octadecyloxyethyl sulfate, $C_{18}H_{37}OCH_2CH_2OSO_3Na$; and sodium 1-

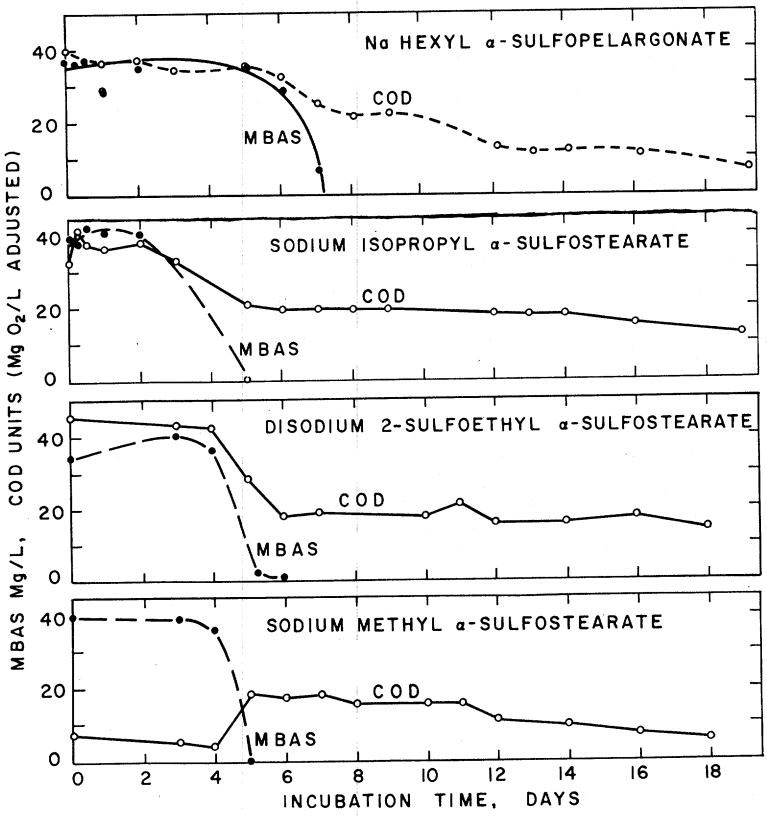

FIG. 4. *Metabolism of α-sulfo fatty acid esters as shown by decrease in COD and MBAS. COD units are adjusted to ppm of detergent.*

TABLE 1. *Comparison of the metabolism of alcohol sulfates, ether alcohol sulfates, and α-sulfo esters*

Detergent	Chemical oxygen demand (mg of O_2 per liter)			Reduction avg
	Theoretical	Lowest	Avg low	
				%
LAS	93.6	4.8	18.8	80
Hydrogenated tallow alcohol sulfates	92.2	0.0	0.9	99
Sodium 9,10-dichlorooctadecyl sulfate	76.1	8.9	19.4	75
Sodium oleyl sulfate	91.6	3.8	4.1	96
Sodium octadecyl-oxyethyl sulfate[a]	90.6	5.7		94
Sodium hexadecyloxypropyl sulfate[a]	89.0	19.6		78
Sulfated polyoxypropylated hexadecanol[a]	89.9	35.3		61
Sodium 1-ethyl-2-hexadecyl-oxyethyl sulfate[a]	90.6	12.2		87
Sodium hexyl α-sulfopelargonate	79.9	19.3	21.0	74
Sodium methyl α-sulfostearate	91.8	13.5	15.1	84
Sodium isopropyl α-sulfostearate	91.1	15.4	24.2	74
Disodium 2-sulfoethyl α-sulfostearate	71.8	16.5	22.0	69

[a] Single experiment.

ethyl-2-hexadecyloxyethyl sulfate, $C_{16}H_{33}OCH_2$-$CH(C_2H_5)OSO_3Na$. The ethylene oxide adduct was the most rapidly and completely metabolized. The persistence of MBAS beyond the almost complete disappearance of COD was due to surface-active matter that could be removed by centrifugation. The COD values showed the oxypropyl adducts to be the most resistant. Nearly 40% of the sulfated polyoxypropylated hexadecanol remained at the end of the test. This was approximately the residue to be expected if only the hexadecyl portion were degraded.

Results with the α-sulfo esters are shown in Fig. 4. Low COD values for sodium methyl α-sulfostearate for the first 4 days were due to limited solubility. The MBAS values showed a disappearance of surface-active properties in 5 to 7 days. The COD values, however, showed the persistence of a small amount of resistant organic matter more than 1 week later.

The COD results (Table 1) represent several runs on each compound, except for the ether alcohol sulfates. Except for LAS, all were with disacclimated inocula. The alcohol sulfates were the most rapidly and completely metabolized. The α-sulfo esters were metabolized at about the same rate and to about the same extent as was LAS. Resistant residues in the biodegradation of ether alcohol sulfates appeared to be related to the oxyalkyl content.

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